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Exposure to the Selective κ-Opioid Receptor Agonist Salvinorin A Modulates the Behavioral and Molecular Effects of Cocaine in Rats

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Stress and chronic exposure to drugs of abuse can trigger addictive and depressive disorders. Both stimuli increase activity of dynorphin, a neuropeptide that acts at κ-opioid receptors (KORs). In humans, KOR agonists cause dysphoria, raising the possibility that dynorphin modulates the depressive-like effects of stress and chronic drug use. We examined if KOR activation alters sensitivity to stimulant drugs by assessing the effects of the selective KOR agonist, salvinorin A (SalvA), on cocaine-induced locomotor activity and c-Fos expression. Acute administration of SalvA blocked the locomotor-stimulant effects of cocaine, whereas repeated SalvA together with concomitant exposure to activity testing chambers potentiated the locomotor response to a cocaine challenge. In contrast, repeated SalvA administered in home cages rather than the activity chambers failed to potentiate the locomotor response to a cocaine challenge. One potential explanation for these findings is that activation of KORs disrupts context conditioning: acute locomotor responses to SalvA alone did not fully habituate with repeated testing in the activity chambers. The effects of SalvA on locomotor activity paralleled its effects on cocaine-induced c-Fos expression in the dorsal striatum: acute SalvA attenuated cocaine-induced c-Fos, whereas repeated SalvA potentiated it when administered in the activity chambers but not the home cage. Acute SalvA also blocked the locomotor stimulant effects of the D1 receptor agonist SKF 82958, whereas repeated SalvA potentiated these effects when administered in the activity chambers. These findings suggest that SalvA regulates the stimulant effects of cocaine through interactions with D1 receptor-mediated signaling in the dorsal striatum.
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INTRODUCTION

The locomotor-stimulant and rewarding effects of cocaine are primarily due to inhibition of the dopamine (DA) reuptake transporter and increased extrasynaptic levels of DA in the dorsal striatum and nucleus accumbens (NAc) (Ritz *et al*, 1987; Wise and Bozarth, 1987; Di Chiara and Imperato, 1988). However, these brain regions are critical integrators of sensori-motor, affective, and cognitive information, and therefore the behavioral effects of cocaine are determined not only by its pharmacological properties, but also the physiological and contextual states of the animal (Barrett, 1987; Falk and Feingold, 1987). For example, prior drug experience, stress and the environment in which cocaine is administered have been shown to modulate behavioral responses to acute and chronic cocaine (Robinson and Berridge, 1993; Shaham *et al*, 2003; Badiani and Robinson, 2004; Todtenkopf and Carlezon, 2006). These factors are thought to contribute to the development and maintenance of addiction (Koob and Le Moal, 1997; Lu *et al*, 2003). Chronic psychostimulant administration and stress can elicit depressive-like states (Kessler, 1997; Markou *et al*, 1998; Goussakov *et al*, 2006), which have been shown to increase anxiety, cocaine craving, and relapse to drug taking (Koob *et al*, 1989; Erb *et al*, 1996; Sinha *et al*, 1999; Covington and Miczek, 2001).

Both stress and repeated administration of psychostimulants increase activity of the neuropeptide dynorphin (Smiley *et al*, 1990; Hurd *et al*, 1992; Spangler *et al*, 1993; McLaughlin *et al*, 2003; Shirayama *et al*, 2004), the endogenous ligand for the κ-opioid receptor (KOR) (Chavkin *et al*, 1982). KOR-specific agonists have depressive-like effects in rodents and humans (Pfeiffer *et al*, 1986; Todtenkopf *et al*, 2004; Carlezon *et al*, 2006), suggesting that aversive states associated with cocaine withdrawal and stress might be due, in part, to activation of KORs. A substantial body of literature demonstrates that acute or repeated treatment of rats with KOR agonists reduces the behavioral effects of psychostimulants (Heidbreder *et al*,

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1995; Gray *et al*, 1999; Schenk *et al*, 1999; Mello and Negus, 2000), although the role of endogenous dynorphin is less clear (Negus *et al*, 1997; Kuzmin *et al*, 1998). However, there is increasing evidence that prolonged or prior exposure to KOR agonists can potentiate the effects of cocaine (Heidbreder *et al*, 1998; McLaughlin *et al*, 2003, 2006; Negus, 2004). KOR agonists also have dissociative effects in humans (Pfeiffer *et al*, 1986) and can inhibit spatial working memory in rats (McDaniel *et al*, 1990). Although less is known about these context-dependent effects of KOR activation, they may impact behavioral responses to psychostimulants that contribute to addiction.

Salvinorin A (SalvA) is a natural psychoactive compound from the leaves of the mint *Salvia divinorum* that is a potent and highly selective KOR agonist (Roth *et al*, 2002). Binding and functional studies demonstrated that SalvA has greater efficacy than the prototypical KOR agonists U-50488H and U-69593 (Chavkin *et al*, 2004; Munro *et al*, 2005). Thus, SalvA is a valuable compound with which to examine interactions between KORs and DA in the striatum and NAc. In addition *S. divinorum* is used in spiritual practices in certain cultures in Mexico, and globally *S. divinorum* and SalvA are becoming increasingly popular as recreational hallucinogens. Humans report psychotropic (and often psychotomimetic) effects of the drug, consistent with its selectivity for KORs (Siebert, 1994; Yan and Roth, 2004). SalvA is currently marketed on the Internet and the fact that it is readily available implies that it is an innocuous substance. However, the long-term effects of exposure to SalvA are not known, nor have interactions with other drugs of abuse been reported.

In the dorsal striatum and NAc, KORs inhibit neuronal activity and neurotransmitter release and are primarily located on presynaptic dopaminergic, GABAergic, and glutamatergic afferents, although there is some evidence for localization on medium spiny output neurons (Arvidsson *et al*, 1995; Svingos *et al*, 1999; Meshul and McGinty, 2000; Hjelmstad and Fields, 2003). Acute administration of SalvA decreases extracellular concentrations of DA in the dorsal striatum (Zhang *et al*, 2005) and NAc (Carlezon *et al*, 2006). Thus KORs are localized in areas where they might modulate locomotor, affective, and cognitive effects of cocaine. However, relatively little is known about plasticity of KORs—or the neural circuits in which they are embedded—after repeated activation that could occur with chronic drug use, stress, or recreational use of *S. divinorum*. The present studies were designed to examine the basic mechanisms of KOR modulation of striatal function as well as the timely issue of potential co-morbidity of *S. divinorum* use and addiction to psychostimulants.

MATERIALS AND METHODS

Animals

A total of 286 male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) were used in this study. Rats weighed 300–350 g at the time of the experiments and were maintained on a 12 h light/dark (7 a.m. to 7 p.m.) cycle with *ad libitum* access to food and water except during testing. Experiments were conducted in accordance with the 1996 National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and McLean Hospital policies.

Drugs

SalvA was provided by Dr David Lee (McLean Hospital, Belmont, MA). The drug was extracted and purified according to established methods (Lee *et al*, 2005). The samples used for testing in this report were determined by high-performance liquid chromatography to be $>99\%$ pure. SalvA was dissolved in a vehicle of 75% dimethyl sulfoxide (DMSO) plus 25% distilled water. Cocaine hydrochloride and (±)-7-OH-DPAT hydrobromide (7-OH-DPAT; Sigma-Aldrich, St Louis, MO) were dissolved in 0.9% saline (NaCl). Chloro-APB hydrobromide (SKF 82958; Sigma-Aldrich) was dissolved in distilled water. Drugs and their respective vehicles were administered by intraperitoneal (i.p.) injection in a volume of 1 ml/kg. Where applicable, doses refer to the salt form of the drug.

Locomotor Activity Tests

All rats were used in experiments designed to test the effects of acute and repeated administration of SalvA on basal and cocaine- or DA receptor agonist-induced locomotor activity. In experiments testing acute effects of SalvA on locomotor activity, rats were placed in automated $43.2 \times 43.2 \times 30.5$ cm ($l \times w \times h$) activity chambers (MED Associates, St Albans, VT) for a 1 h habituation session on day 0. Rats were then divided into various treatment groups such that the average total distances traveled in each group during the habituation session did not significantly differ. On day 1, rats were placed into the activity chambers for 1 h. Rats were then removed from the chambers, weighed, and injected first with SalvA or vehicle followed 5 min later with cocaine, SKF 82958, 7-OH-DPAT, or vehicle. Rats were then placed back in the activity chambers for an additional 2 h. In experiments testing the effects of repeated administration of SalvA on basal and cocaine- or DA receptor agonist-induced locomotor activity, rats were placed in the activity chambers for a 1 h habituation session on day 0. Rats were divided into treatment groups as above. On days 1–5 and 8, rats were weighed and injected with SalvA or vehicle and placed in the activity chambers for 3 h. Rats remained untreated and in their home cages on days 6 and 7. On day 9, rats were placed in the activity chambers for 1 h. Rats were then removed from the chambers, weighed, and injected with cocaine, SKF 82958, 7-OH-DPAT, or vehicle. Rats were then placed back in the activity chambers for an additional 2 h. In these experiments, repeated exposure to the activity chambers rendered them a 'familiar' environment. To test the effect of repeated SalvA administered in the home cage on subsequent cocaine-induced locomotor activity, rats were treated with SalvA or vehicle on days 1–5 and immediately returned to their home cages. On day 8, rats were habituated for 1 h in the activity chambers, removed, injected with SalvA or vehicle, and returned to their home cages. On day 9, rats were placed in the activity chambers for 1 h. Rats were then removed from the chambers, weighed, and injected with cocaine or vehicle. Rats were then placed back in the activity chambers for an additional 2 h. This scheme was designed to provide a

similar structure to the acute and prior, repeated SalvA experiments, but with minimal exposure to the activity chambers until the cocaine challenge day, thus rendering the activity chambers a 'novel' environment.

The total number of activity counts (photocell beam breaks) during the test sessions was quantified in 15-min bins and converted to Distance Traveled in cm. Differences among treatment groups were analyzed using a one-way ANOVA (for total distance traveled) and a two-way ANOVA (treatment × time) with repeated measures on time. Significant effects were analyzed using *post hoc* Fisher's protected *t*-tests.

Immunohistochemistry

For analysis of c-Fos induction in response to acute or repeated SalvA treatments, a subset of rats (90) was killed immediately after completion of respective locomotor tests. The rats were overdosed with pentobarbital (130 mg/kg, i.p.) and transcardially perfused with ice-cold 0.9% saline (NaCl) followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS, pH 7.4). The fixed brains were removed and postfixed for 3 days at 4°C, then transferred to 20% glycerol in 50 mM phosphate buffer (PB; pH 7.4) at 4°C until saturation (⩾24 h). Coronal sections (40 μM) were cut on a freezing microtome and stored in cryoprotectant (50% ethylene glycol, 20% glycerol, 10 mM PB, 150 mM NaCl, 3 mM KCl) at −20°C until Immunohistochemistry (IHC) was performed. For c-Fos IHC, free-floating sections were rinsed 3 × 10 min in 0.01 M Tris-buffered saline, pH 7.4 (TBS) and then blocked for 2 h at room temperature in AB media (0.3% Triton X-100, 2% normal goat serum (Invitrogen, Carlsbad, CA), and 1% bovine serum albumin (Sigma) in 0.01 M TBS). The sections were then incubated on a shaker overnight at room temperature with a polyclonal antibody made in rabbit directed against c-Fos (PC38T, Calbiochem, La Jolla, CA), diluted 1:10 000 in AB media. The following day, sections were rinsed 3 × 10 min in 0.01 M TBS and incubated for 1 h at room temperature in biotinylated goat anti-rabbit immunoglobulin G secondary antibody (Vector Laboratories, Burlingame, CA) diluted 1:200 in AB media. Following 3 × 10 min rinses in 0.01 M TBS, sections were incubated with avidin-biotin-peroxidase complex (Vectastain ABC Elite kit; Vector Laboratories) for 30 min at room temperature. After 3 × 5 min rinses in 0.01 M TBS, sections were reacted with 0.05% 3,3′-diaminobenzidine tetrahydrochloride containing 0.01% H_2O_2 (Sigma) for 10 min. Rinsing in 0.01 M phosphate buffer terminated the reaction.

To quantify the number of c-Fos-positive nuclei in brain regions of interest, still images were taken at × 5 using a Zeiss Axioscope 2 (Zeiss, Oberkochen, Germany) and a digital camera (AxioCam, Zeiss) interfaced with a Macintosh G4 computer. Images from each brain region of interest were taken from three sections per treatment corresponding approximately to bregma +1.60 mm (NAc, dorsal striatum, and PfCx) and −2.80 (LA and CeA) (Paxinos and Watson, 1986). Digital photos were analyzed with Image J software for Macintosh (NIH, Bethesda, MD; http://rsb.info.nih.gov/ij/) by an observer blind to the treatment groups. Each brain region of interest was outlined using anatomical markers. The area of the outlined region was measured using arbitrary units (pixels per inch) and was used to calculate the density of c-Fos staining in each section (density = number of c-Fos nuclei per area). A threshold intensity and size range for c-Fos-positive nuclei was set so that all positively labeled cells in a region of interest were counted and signal due to background labeling was not. These parameters were determined separately for each experiment and were used for all analyses within an experiment. In experiments examining effects of SalvA on cocaine-induced c-Fos, differences among treatment groups were analyzed using a one-way ANOVA (c-Fos density). Significant effects were analyzed using *post hoc* Fisher's protected *t*-tests. In experiments examining effects of acute SalvA on c-Fos expression in different brain regions, two-tailed unpaired Student's *t*-tests were used for each brain region.

RESULTS

Acute SalvA Attenuates the Locomotor Stimulant Effects of Cocaine

To determine the immediate effects of KOR activation on the locomotor stimulant effects of cocaine, SalvA was administered 5 min prior to cocaine and locomotor activity was monitored for 2 h. Over the course of the 2 h (Figure 1a), a two-way repeated measures ANOVA revealed a significant treatment × time interaction ($F_{24,248} = 4.57$; $P < 0.001$). *Post hoc* analyses showed that, in the 1 h after drug administration, rats treated with vehicle (75% DMSO) plus cocaine had significantly greater locomotor activity compared to rats treated with vehicle plus saline, and this was significantly reduced by SalvA pretreatment. The total distance traveled over the first hour after drug administration (Figure 1b) depended on treatment ($F_{3,31} = 8.09$; $P < 0.001$): SalvA blocked cocaine-stimulated locomotor activity, and locomotor activity in rats treated with SalvA plus cocaine did not differ from that of rats treated with either vehicle or SalvA plus saline.

Locomotor Response to Repeated SalvA

To examine how repeated activation of KORs affects behavior over time, we treated rats with SalvA or vehicle once a day for 5 days (d1–d5) and measured locomotor activity for 3 h after each injection. To test for the development of tolerance or sensitization to repeated activation of KORs, we then treated the rats with SalvA or vehicle 3 days after the last of the 5-d regimen (d8) and measured locomotor activity for 3 h. We previously demonstrated that SalvA reduces extracellular DA levels in the NAc for ⩾2 h (Carlezon *et al*, 2006). Thus, we chose to assess locomotor activity for 3 h in order to be able to detect any rebound effects on behavior after DA levels had recovered. A two-way repeated measures ANOVA (treatment × time) revealed that over the 6 treatment days, the effect of SalvA on locomotor activity depended on treatment ($F_{1,76} = 12.57$; $P < 0.001$) and time ($F_{5,380} = 18.40$; $P < 0.001$) (Figure 2a). Except for d2, SalvA significantly increased the total distance traveled each day compared to vehicle. The effects of SalvA on locomotor activity over the 3 h test sessions depended on interactions between treatment and

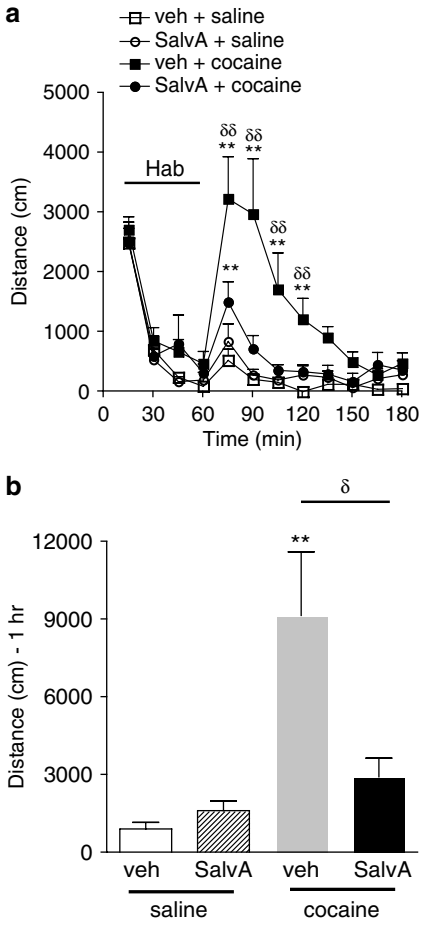


Figure 1 Effect of acute salvinorin A (SalvA) on cocaine-induced locomotor activity. (a) Time course of locomotor activity (distance in cm ± SEM) in response to vehicle (veh; 75% dimethyl sulfoxide (DMSO) or SalvA (2 mg/kg, i.p.) plus saline or cocaine (10 mg/kg, i.p.)). Rats were habituated (Hab) to the activity chambers for 1 h prior to veh or SalvA, and cocaine was administered 5 min later. Significant differences in locomotor activity among treatment groups are as follows: $^{**}P<0.01$ compared to veh + saline; $^{\delta\delta}P<0.01$ compared to SalvA + cocaine, Fisher's protected *t*-tests, 8–9 rats per group. (b) Cumulative locomotor activity (total distance in cm ± SEM) in the first hour after SalvA (or veh) plus cocaine (or saline) treatment. $^{**}P<0.01$ compared to veh + saline; $^{\delta}P<0.05$ comparing groups under bar.

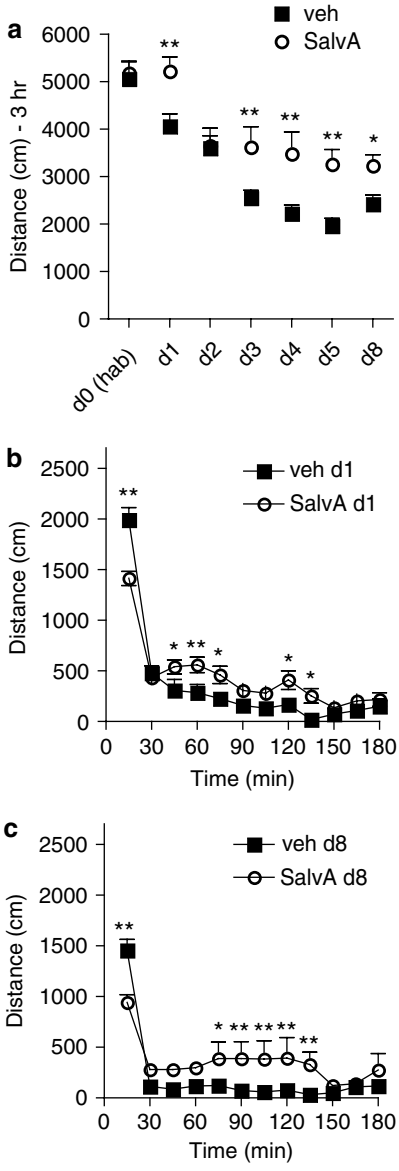


Figure 2 Effect of salvinorin A (SalvA) on basal locomotor activity. (a) Cumulative locomotor activity (total distance in cm ± SEM) is increased in rats treated with SalvA (2 mg/kg per day, i.p.) compared to veh-treated rats. day 0, habituation (d0, (hab)) reflects cumulative locomotor activity for 1 h, whereas d1–d5, and d8 reflect locomotor activity for 3 h. (b, c) Time course of locomotor activity (distance in cm ± SEM) over the 3 h test period for treatment day 1 (d1), (b) and day 8 (d8), (c) shows that SalvA decreases locomotor activity in the first 15 min but then maintains elevated locomotor activity for much of the remaining 3 h test session. $^{*}P<0.05$; $^{**}P<0.01$ compared to veh, Fisher's protected *t*-tests, 39 rats per group.

time for both d1 ($F_{11,825}=5.88$; $P<0.001$) and d8 ($F_{11,836}=4.71$; $P<0.001$) (Figure 2b and c). *Post hoc* analyses revealed that, on both d1 and d8, SalvA significantly reduced locomotor activity in the first 15 min after drug injection, but then maintained a low level of heightened activity for the next 2 h (Figure 2b and c). This differed significantly from the response of vehicle-treated rats, which showed an initial burst of activity in the first 15 min and then quickly habituated to little or no movement for the remainder of the test session.

Effects of Prior Exposure to Repeated SalvA on the Locomotor Stimulant Effects of Cocaine

Considering that both stress and chronic cocaine increase dynorphin levels in the striatum and increase the likelihood of future drug seeking, we tested the impact of repeated administration of SalvA on subsequent cocaine-induced increases in locomotor activity. Rats were treated with SalvA as described above: on days 1–5 and on day 8, rats were injected with SalvA or vehicle and placed in activity chambers for 3 h. On day 9, rats were placed in the activity chambers for 1 h to habituate and were then injected with cocaine or saline and returned to the activity chambers for 2 h. The effects of SalvA on cocaine-induced locomotor activity during this 2 h time period depended on an interaction between treatment and time ($F_{24,352}=5.00$; $P<0.001$) (Figure 3a). *Post hoc* analyses revealed that rats treated previously with either vehicle or SalvA showed significantly

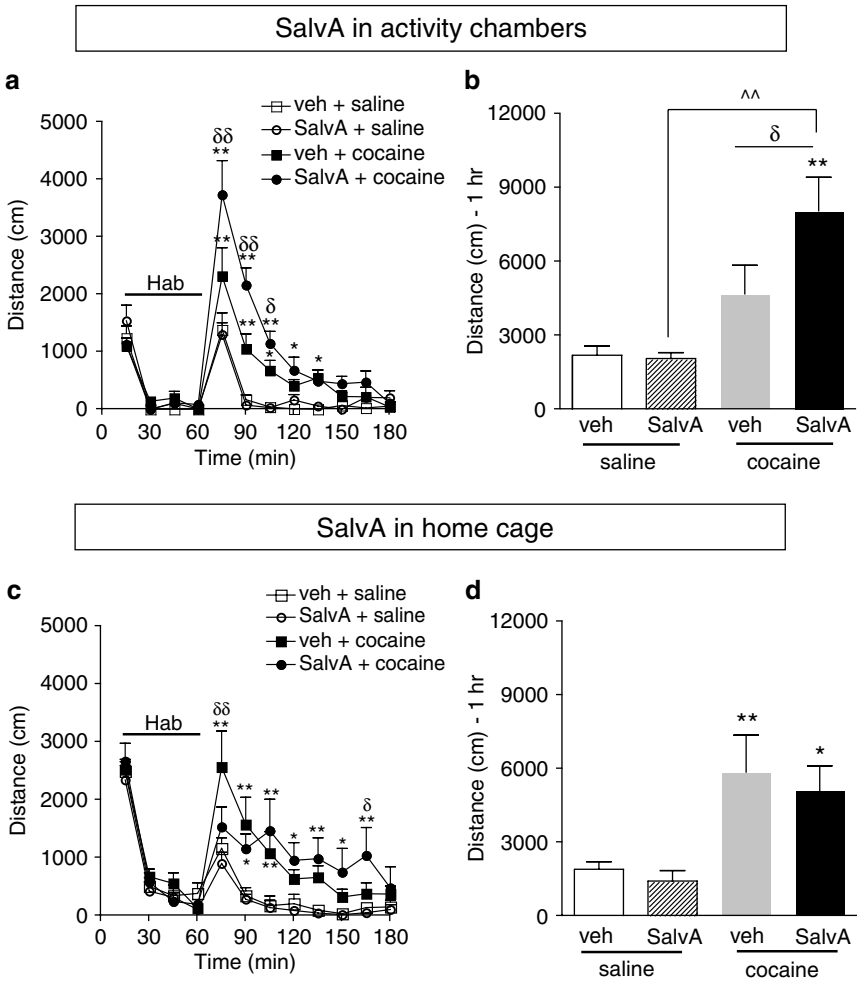


Figure 3 Effect of exposure to repeated salvinorin A (SalvA) on cocaine-induced locomotor activity. (a) Time course of locomotor activity (distance in cm ± SEM) in response to a saline or cocaine (10 mg/kg, i.p.) challenge given on treatment day 9. On days 1–5 and 8, rats were treated for 1 day with either veh (75% dimethyl sulfoxide (DMSO)) or SalvA (2 mg/kg, i.p.) and placed in the activity chambers for 3 h after each drug injection. (b) Cumulative locomotor activity (total distance in cm ± SEM) in the first hour after saline or cocaine challenge on day 9. Rats were treated on days 1–5 and 8 as described above in (a), $^{\wedge\wedge}P<0.01$. (c) Time course of locomotor activity (distance in cm ± SEM) in response to a saline or cocaine (10 mg/kg, i.p.) challenge given on treatment day 9. On days 1–5 and 8, rats were treated 1xday with either veh (75% DMSO) or SalvA (2 mg/kg, i.p.) and returned immediately to their home cages after each drug injection. (d) Cumulative locomotor activity (total distance in cm ± SEM) in the first hour after saline or cocaine challenge on day 9. Rats were treated on days 1–5 and 8 as described above in (c). $^{*}P<0.05$, $^{**}P<0.01$ compared to veh + saline; $^{\delta}P<0.05$, $^{\delta\delta}P<0.01$ comparing veh + cocaine and SalvA + cocaine; $^{\wedge}P<0.05$ comparing SalvA + saline and SalvA + cocaine; Fisher's protected *t*-tests, 8–15 rats per group.

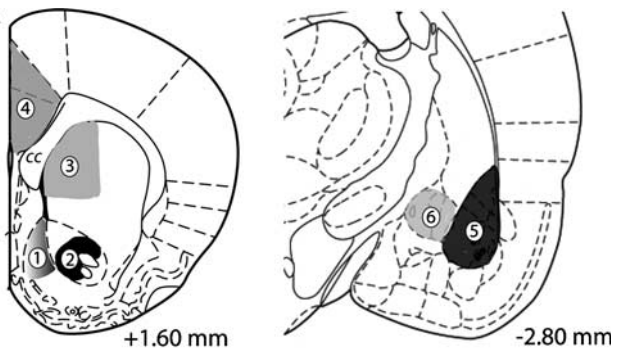
increased locomotor activity after a cocaine challenge compared to rats given a saline challenge. However, rats treated previously with SalvA had significantly greater locomotor activity in response to cocaine than those treated previously with vehicle. The total distance traveled in the first hour after cocaine or saline injection depended on treatment ($F_{3,32}=5.36$; $P<0.05$) (Figure 3b). Interestingly, cocaine significantly induced locomotor activity in rats previously treated with SalvA, but not vehicle, compared to controls.

The total distance traveled 1 h after cocaine administration in rats previously treated with repeated vehicle in the activity chambers (Figure 3b; 4673 cm ± 1158 SEM) was markedly less than the distance traveled 1 h after cocaine administration in rats acutely pre-treated with vehicle (Figure 1b; 9132 cm ± 2453 SEM). The primary difference between these two experiments is repeated exposure to the activity chambers and daily drug injections (Figure 3b) *vs* brief exposure to the activity chambers and a single day of drug injections (Figure 1b). This led us to hypothesize that repeated activation of KORs might be preventing the normal (and well described) process of habituation that most likely contributes to the decreased effects of psychostimulants in rats treated in familiar environments (Kiyatkin, 1992; Badiani *et al*, 1995). To test this hypothesis, we treated rats with SalvA or vehicle on d1–d5 and d8 and immediately returned them to their home cages. On day 9, rats were placed in the activity chambers for 1 h to habituate and were then injected with cocaine or vehicle and returned to the activity chambers for 2 h. The effects of prior, repeated SalvA administered in the home cages on cocaine-induced locomotor activity during this 2 h time period depended on an interaction between treatment and time ($F_{24,256}=1.76$; $P<0.05$) (Figure 3c). *Post hoc* analyses revealed that cocaine significantly increased locomotor activity in rats previously treated in their home cages with vehicle or SalvA. However, rats previously treated with SalvA showed sustained increases in locomotor activity throughout the 2 h test session,

whereas rats previously treated with vehicle had significantly increased locomotor activity for the first 45 min after cocaine injection. Furthermore, cocaine-induced locomotor activity was significantly decreased in the first 15 min in rats previously treated with SalvA. The total distance traveled in the first hour after cocaine or saline injection depended on treatment ($F_{3,32} = 5.96$; $P < 0.05$) (Figure 3d). Cocaine significantly increased locomotor activity in rats that had been pretreated with either vehicle or SalvA in their home cages, and there was no difference in the level of cocaine-induced locomotor activity between these two pretreatment groups.

SalvA Induces c-Fos Expression in Limbic Brain Regions

Stimulus-induced c-Fos expression is indicative of either direct or indirect activation of cAMP and/or Ca^{2+} second messenger pathways (Sheng *et al*, 1990) and is often considered a marker of neuronal activation and plasticity (Morgan and Curran, 1991). Given the psychotomimetic and hallucinogenic properties of SalvA, as well as its effects on locomotor activity, we examined whether acute SalvA treatment induced c-Fos expression in several brain regions implicated in mood regulation (NAc shell), cognition (prefrontal cortex; PfCx, hippocampus), emotionality (central and lateral amygdala; CeA and LA, respectively), and motor control (dorsal striatum, NAc core) (Figure 4a). We found that rats treated acutely with SalvA had significantly more c-Fos-positive nuclei in the NAc shell, PfCx, LA, and CeA than control rats treated with vehicle (Figure 4b). There was a trend toward significant induction in the dorsal striatum ($P = 0.058$, Student's *t*-test) and no effect of SalvA on c-Fos expression in the NAc core (Figure 4b). There was no evidence of c-Fos-positive nuclei in the dorsal hippocampus (at bregma −2.80 mm) in either control or SalvA-treated rats (data not shown).



b

	Veh	SalvA
NAc shell (1)	1.62 (±0.23)	3.57 (±0.63)*
NAc core (2)	1.13 (±0.20)	1.72 (±0.46)
Dorsal striatum (3)	0.34 (±0.08)	1.00 (±0.30)^{t}
PfCx (4)	1.23 (±0.12)	2.75 (±0.55)*
LA (5)	0.27 (±0.04)	0.69 (±0.14)*
CeA (6)	0.49 (±0.14)	1.52 (±0.13)**

Figure 4 Effect of acute salvinorin A (SalvA) on c-Fos expression in rat brain. (a) Representative schematics from rat brain atlas (Paxinos and Watson, 1986) showing brain regions analyzed for c-Fos-positive nuclei. Regions at bregma + 1.60 include nucleus accumbens (NAc) shell (1), NAc core (2), dorsal striatum (3), and prefrontal cortex (PfCx) (4). Regions at bregma −2.80 include lateral amygdala (LA) (5) and central nucleus of the amygdala (CeA) (6). (b) Table reporting quantification of the density of c-Fos-positive nuclei in response to veh (75% dimethyl sulfoxide (DMSO)) or SalvA (2 mg/kg, i.p.). Data are expressed as mean c-Fos density per region ± SEM in parentheses. *$P < 0.05$, **$P < 0.01$; t = trend ($p = 0.058$), Students *t*-tests compared to veh for each respective brain region, 6–9 rats per group.

Interactions between Acute SalvA and Cocaine on c-Fos Expression

Cocaine induces c-Fos in several brain regions implicated in motor control and motivational state (Harlan and Garcia, 1998), but the effects of SalvA on c-Fos expression are not known. We examined the regulation of c-Fos by SalvA and cocaine in the dorsal striatum, NAc shell, and NAc core (Figure 5). We found treatment-dependent effects on the number of c-Fos-positive cells in the dorsal striatum ($F_{3,20} = 11.20$; $P < 0.001$) (Figure 5a and c), NAc shell ($F_{3,20} = 3.47$; $P < 0.05$), and the NAc core ($F_{3,20} = 3.94$; $P < 0.05$) (Figure 5b and c). Specifically, cocaine significantly increased c-Fos expression in the dorsal striatum ($P < 0.01$) and NAc core ($P < 0.05$), but not the NAc shell, although there was a trend for cocaine to significantly increase c-Fos expression in the NAc shell. Consistent with the ability of SalvA to decrease cocaine-stimulated locomotor activity, SalvA reduced cocaine-induced c-Fos expression in the dorsal striatum ($P < 0.05$), but not in the NAc shell or NAc core.

Cocaine-Induced c-Fos after Exposure to Repeated SalvA

To further test the hypothesis that interactions between KOR and DA signaling in the striatum mediate the locomotor response to cocaine, we examined c-Fos expression in rats treated repeatedly with SalvA in the activity chambers and subsequently challenged with cocaine. We found treatment-dependent effects on the number of c-Fos-positive cells in the dorsal striatum ($F_{3,31} = 8.38$; $P < 0.001$), NAc shell ($F_{3,32} = 4.87$; $P < 0.01$), and NAc core ($F_{3,32} = 16.39$; $P < 0.0001$) (Figure 6). In each brain region, cocaine significantly increased the number of c-Fos-positive cells, regardless of pretreatment with vehicle or SalvA. In the dorsal striatum, c-Fos expression was significantly higher in rats treated repeatedly with SalvA in the activity chambers and challenged with cocaine compared to rats treated repeatedly with vehicle and challenged with cocaine (Figure 6a and c). To determine whether cocaine-induced c-Fos in the striatum is modulated by contextual experience, we measured c-Fos expression in rats treated repeatedly with SalvA in the home cage and subsequently challenged with cocaine in the activity chambers (Figure 7). We found treatment-dependent effects in the dorsal striatum ($F_{3,20} = 11.18$; $P < 0.001$), NAc shell ($F_{3,20} = 3.14$; $P < 0.05$), and NAc core ($F_{3,20} = 12.54$; $P < 0.001$). Cocaine significantly increased c-Fos in each brain region, but in contrast to rats that received SalvA in the activity chambers, there was no difference in c-Fos expression in the dorsal striatum of rats treated repeatedly with SalvA or vehicle in the home cage and challenged with cocaine (Figure 7a and c). Interestingly, these findings in the dorsal striatum correlate with the respective locomotor responses to cocaine (Figure 3).

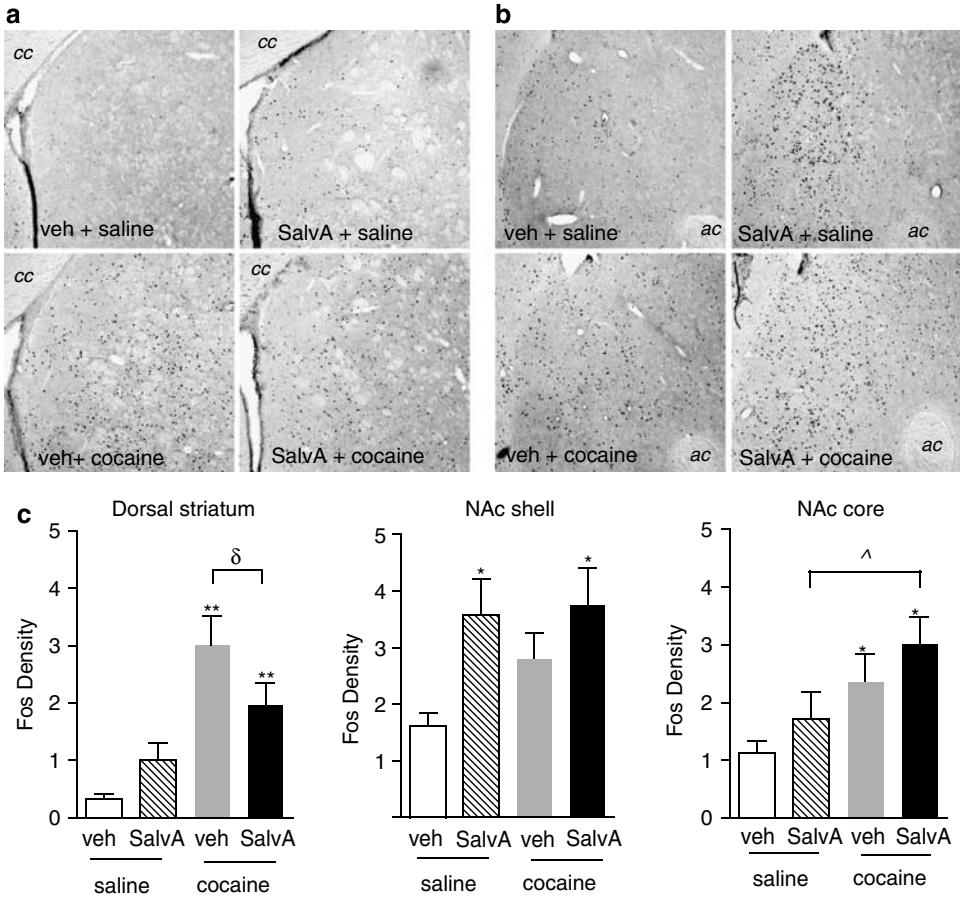


Figure 5 Effect of acute salvinorin A (SalvA) on cocaine-induced c-Fos expression in the striatum. (a, b) Representative images of c-Fos immunoreactivity from the dorsal striatum (a) and nucleus accumbens (NAc) (b). (c) Quantification of the density of c-Fos-positive nuclei in the dorsal striatum, NAc shell, and NAc core in response to veh or SalvA (2 mg/kg, i.p.) and saline or cocaine (10 mg/kg, i.p.; see 'Materials and Methods'). Rats were killed 2 h after drug administration. $^{*}P<0.05$, $^{**}P<0.01$ compared to veh + saline; $^{\delta}P<0.05$ comparing veh + cocaine and SalvA + cocaine; $^{\wedge}P<0.05$ comparing SalvA + saline and SalvA+cocaine; Fisher's protected *t*-tests, six rats per group.

Effects of SalvA on DA Receptor Agonist-Induced Locomotor Activity

To further address the possibility that KOR activation might mediate cocaine-stimulated locomotor activity through interactions with DA receptor signaling, we administered SalvA either acutely or repeatedly in the activity chambers and measured the effects on DA receptor agonist-induced locomotor activity. The effect of acute pretreatment with SalvA on the total distance traveled in the first hour after DA receptor agonist treatment (Figure 8a) depended on treatment ($F_{7,57}=2.69$; $P<0.05$). *Post hoc* analyses revealed that, in control rats, the DA D1 receptor agonist SKF 82958 (0.1 mg/kg) significantly increased locomotor activity whereas the DA D2/D3 receptor agonist 7-OH-DPAT did not significantly increase locomotor activity at the lower dose (1.0 mg/kg) but caused a trend toward an increase ($P=0.069$) at the higher dose (3.0 mg/kg). Acute SalvA pretreatment blocked SKF 82958-induced locomotor activity and had no effect on 7-OH-DPAT-induced activity. The effect of repeated SalvA treatment—with concomitant exposure to the activity chambers—on the total distance traveled in the first hour after a DA receptor agonist challenge (Figure 8b) depended on treatment ($F_{7,58}=2.58$; $P<0.05$). In this case, the higher dose of 7-OH-DPAT (3.0 mg/kg) significantly increased locomotor activity in control rats treated with vehicle. Neither the lower dose of 7-OH-DPAT (1.0 mg/kg) nor SKF 82958 (0.1 mg/kg) had any effect on locomotor activity compared to control rats treated with vehicle. Repeated treatment with SalvA had no effect on 7-OH-DPAT-induced locomotor activity, but resulted in an increase in the locomotor response to SKF 82958 such that locomotor activity in rats treated with repeated SalvA and challenged with SKF 82958 demonstrated significantly more locomotor activity than vehicle-treated control rats.

DISCUSSION

These studies were designed to test the hypothesis that acute activation of KORs—as might occur during a period of acute stress or consumption of psychostimulants—would attenuate the behavioral effects of cocaine, whereas prior exposure to repeated KOR activation—as might occur after chronic stress or psychostimulant administration—would potentiate the behavioral effects of cocaine. Furthermore, we investigated whether the striatum was a

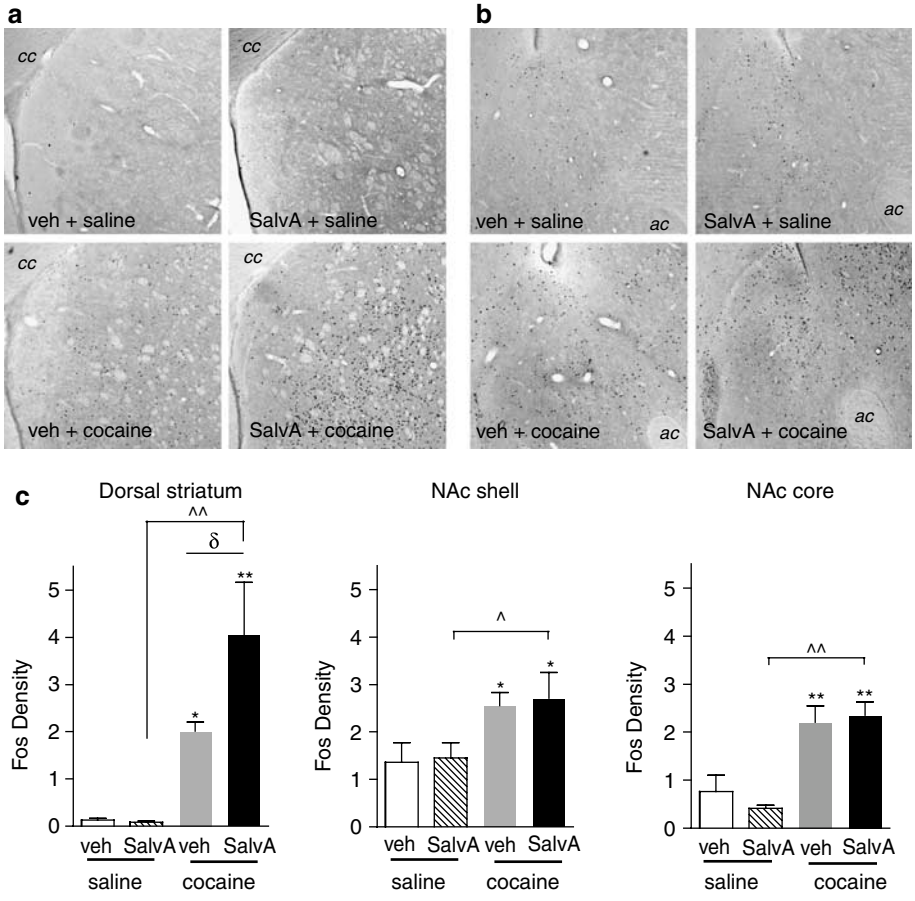


Figure 6 Effect of repeated salvinorin A (SalvA) administered in the activity chambers on cocaine-induced c-Fos expression in the striatum. (a, b) Representative images of c-Fos immunoreactivity from the dorsal striatum (a) and nucleus accumbens (NAc) (b). (c) Quantification of the density of c-Fos-positive nuclei in the dorsal striatum, NAc shell, and Nac core in response to a saline or cocaine (10 mg/kg, i.p.) challenge given on treatment day 9 (see 'Materials and Methods'). Rats were killed 2 h after drug administration. On days 1–5 and 8, rats were treated for 1 day with either veh (75% dimethyl sulfoxide (DMSO)) or SalvA (2 mg/kg, i.p.) and placed in the activity chambers for 3 h after each drug injection. $^{*}P<0.05$, $^{**}P<0.01$ compared to veh + saline; $^{\delta}P<0.05$ comparing veh + cocaine and SalvA + cocaine; $^{\wedge}P<0.05$, $^{\wedge\wedge}P<0.01$ comparing SalvA + saline and SalvA + cocaine; Fisher's protected *t*-tests, six rats per group.

neural substrate for interactions between KOR and DA signaling by examining the regulation of the immediate early gene c-Fos. The rationale for conducting these studies stems from findings that both stress and chronic psychostimulant administration increase dynorphin activity in the striatum (Smiley *et al*, 1990; Hurd *et al*, 1992; Spangler *et al*, 1993; McLaughlin *et al*, 2003; Shirayama *et al*, 2004) and can subsequently increase addictive behaviors (Koob and Le Moal, 1997; Lu *et al*, 2003). As expected, we found that acute SalvA blocked the locomotor stimulant effects of cocaine. In contrast, we found that prior exposure to repeated administration of SalvA potentiated the locomotor response to a subsequent cocaine challenge. After each daily SalvA treatment, locomotor activity was measured and remained elevated above vehicle-treated controls. In a subsequent experiment in which rats were treated repeatedly with SalvA in the home cage without exposure to the activity chambers, we found that the overall locomotor response to a subsequent cocaine challenge was unchanged compared to controls, although there was a significant decrease in the peak effect of cocaine. Together, these findings raise the possibility that SalvA inhibits contextual habituation, either directly or indirectly via simultaneous processes that mask the effects of habituation. The effects of SalvA on cocaine-stimulated locomotor activity may be mediated, at least in part, by the striatum, as cocaine-induced c-Fos expression in the dorsal striatum was reduced by acute SalvA, increased by prior exposure to repeated SalvA given in the activity chambers, and unchanged by prior exposure to repeated SalvA given in the home cages. Furthermore, SalvA itself induced c-Fos in the NAc shell, PfCx, and amygdala, suggesting that these limbic brain regions mediate the motivational and cognitive effects of SalvA. Finally, we found that acute SalvA blocked, and repeated SalvA increased, DA D1 receptor agonist-stimulated locomotor activity, raising the possibility that activation of KORs directly modulates DA receptor signaling in the striatum.

When considered together with previous work (Thompson *et al*, 2000; Carlezon *et al*, 2006), these findings suggest that KORs in the dorsal striatum and NAc can mediate both motivational and locomotor-stimulant effects of cocaine, respectively—perhaps through similar mechanisms. The aversive effects of KOR agonists—including dysphoria and anhedonia—are thought to be mediated by the NAc and typically occur within the first hour after drug administration (Shippenberg and Herz, 1988; Todtenkopf *et al*, 2004;

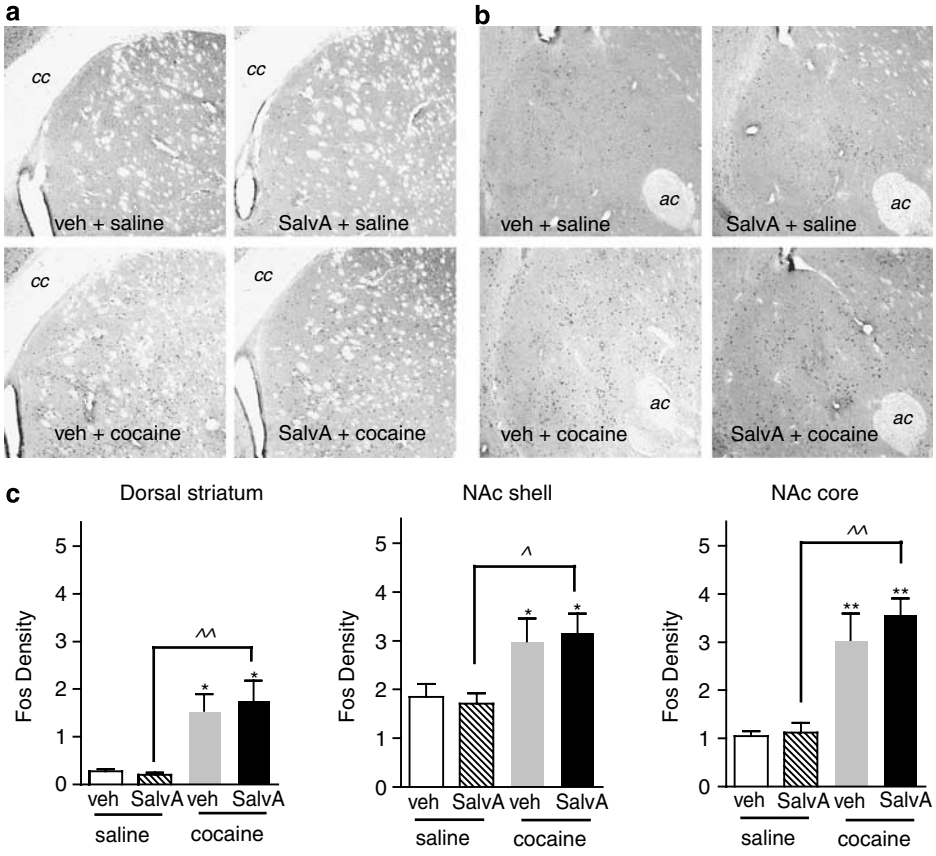


Figure 7 Effect of repeated salvinorin A (SalvA) administered in the home cage on cocaine-induced c-Fos expression in the striatum. (a, b) Representative images of c-Fos immunoreactivity from the dorsal striatum (a) and nucleus accumbens (NAc) (b). (c) Quantification of the density of c-Fos-positive nuclei in the dorsal striatum, NAc shell, and NAc core in response to a saline or cocaine (10 mg/kg, i.p.) challenge given on treatment day 9 (see 'Materials and Methods'). Rats were killed 2 h after drug administration. On days 1–5 and 8, rats were treated for 1 day with either veh (75% dimethyl sulfoxide (DMSO)) or SalvA (2 mg/kg, i.p.) and returned immediately to their home cages after each drug injection. $^{*}P<0.05$, $^{**}P<0.01$ compared to veh + saline; $^{\wedge}P<0.05$, $^{\wedge\wedge}P<0.01$ comparing SalvA + saline and SalvA + cocaine; Fisher's protected *t*-tests, 7–10 rats per group.

Carlezon *et al*, 2006). Our finding that SalvA acutely inhibits cocaine-stimulated locomotor activity is consistent with other studies showing KOR agonist-mediated reduction in the behavioral effects of cocaine and in basal reward function (Gray *et al*, 1999; Schenk *et al*, 1999; Todtenkopf *et al*, 2004; Carlezon *et al*, 2006; McLaughlin *et al*, 2006). Recently it has been demonstrated that prior or repeated activation of KORs can potentiate the rewarding effects of cocaine (Heidbreder *et al*, 1998; Negus, 2004; McLaughlin *et al*, 2006). Our finding that repeated treatment with SalvA combined with repeated exposure to the activity testing chambers potentiates the locomotor response to a subsequent cocaine challenge is consistent with these effects and suggests that the striatum—both dorsal and ventral (NAc)—is a neural substrate. On the surface, this finding appears to contradict a body of literature demonstrating that repeated administration of KOR agonists reduces cocaine-stimulated locomotor activity and attenuates behavioral sensitization to cocaine (Heidbreder *et al*, 1995; Shippenberg *et al*, 1996; Shippenberg and Rea, 1997). In these previous studies, however, KOR agonists were administered in the home cage. Thus, our finding that the maximum effect of cocaine is attenuated in response to a cocaine challenge in rats treated repeatedly with SalvA in the home cage is consistent with this work and highlights the importance of environmental context on behavior. Together, these findings suggest that KOR activation attenuates normal processes of context habituation and can thereby potentiate the stimulant properties of cocaine. Considering that novelty enhances the locomotor stimulant properties of psychostimulants (Kiyatkin, 1992; Badiani *et al*, 1995), the proposed dissociative effects of KOR activation might result in environments remaining novel even after repeated exposure.

Exposure to a novel environment produces a stress response (Badiani and Robinson, 2004), which has been shown to increase locomotor stimulant and reinforcing properties of psychostimulants (Herman *et al*, 1984; Erb *et al*, 1996; Covington and Miczek, 2001; McLaughlin *et al*, 2003). Considering the known role of KORs in mediating responses to stress, SalvA might activate stress pathways that enhance sensitivity to the locomotor stimulant effects of cocaine, thereby overriding the habituation-dependent decrease in cocaine-induced locomotor activity. Thus, rather than a dissociative effect, SalvA might facilitate associative learning between a stressor (KOR activation) and a context (locomotor activity chamber).

The mechanisms by which repeated KOR activation modulates the locomotor stimulant effects of cocaine are

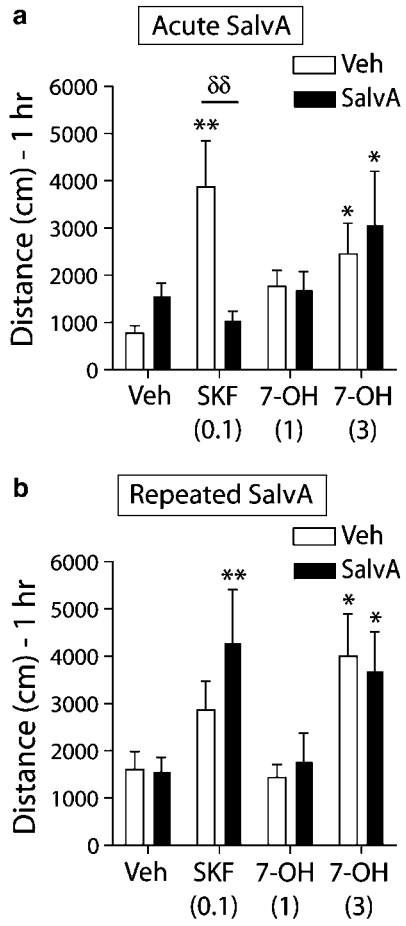


Figure 8 Effects of acute and prior, repeated administration of salvinorin A (SalvA) on dopamine (DA) receptor agonist-induced locomotor activity. (a) Cumulative locomotor activity (total distance in cm ± SEM) in the first hour after SalvA (2 mg/kg, i.p.) or veh (75% dimethyl sulfoxide (DMSO)) plus veh (see 'Materials and Methods'), SKF 82958 (0.1 mg/kg, i.p.), or 7-OH-DPAT (1 and 3 mg/kg, i.p.) treatment. (b) Cumulative locomotor activity (total distance in cm ± SEM) in the first hour after saline or DA agonist challenge on day 9. On days 1–5 and 8, rats were treated for 1 day with either veh (75% DMSO) or SalvA (2 mg/kg, i.p.) and placed in the activity chambers for 3 h after each drug injection. $^{*}P<0.05$, $^{**}P<0.01$ compared to veh + veh; $^{\delta\delta}P<0.01$ comparing groups under bar; Fisher's protected *t*-tests, 8–12 rats per group.

unknown. One potential mechanism is that KORs become desensitized after repeated SalvA administration. As a consequence, the inhibitory effects of KORs on DA release within the striatum would be reduced. In support of a role for receptor desensitization, it has been shown that the KOR agonist U50-488 induces GRK3-dependent receptor phosphorylation and internalization *in vivo* (McLaughlin *et al*, 2004). Furthermore, basal extracellular levels of DA are increased in the NAc after infusion of the KOR antagonist norBNI (Spanagel *et al*, 1992) and in KOR knockout mice (Chefer *et al*, 2005), suggesting that in the absence of functional KORs, DA activity is enhanced. However, KOR desensitization is unlikely in the current study because the daily locomotor response to SalvA did not change, suggesting the function of KORs remains constant.

Alternatively, repeated SalvA might lead to a context-dependent change in the activation and/or sensitivity of DA receptors within the striatum. This could include changes in DA release, DA receptor number, or in coupling of receptors to downstream effector systems. It has been previously shown that exposure of rats to a novel environment does not affect cocaine-induced DA release in the NAc or dorsal striatum (Badiani *et al*, 1998), suggesting that postsynaptic mechanisms might be involved. Our finding that acute SalvA attenuates—whereas repeated SalvA administered in the activity chambers facilitates—the locomotor stimulant effects of a D1 receptor agonist supports this possibility. Previous work has shown that repeated treatment with the KOR agonist U-69593 in the home cage leads to decreased DA D2, but not D1, receptor levels in the dorsal striatum and a decrease in the locomotor stimulant effects of the D2 agonist quinpirole (Izenwasser *et al*, 1998). Although we did not observe an effect of acute or repeated SalvA on D2 receptor agonist-induced locomotor activity, our combined findings suggest that repeated activation of KORs alters DA receptor signaling in a context-dependent manner.

Consistent with previous work (Harlan and Garcia, 1998), we found that cocaine induced c-Fos in both motor (dorsal striatum, NAc core) and limbic (NAc shell) brain regions. SalvA modulates cocaine-induced c-Fos in the dorsal striatum in a manner analogous to its effects on locomotor behavior, suggesting that this region is an important neural substrate for interactions between cocaine and KORs. We also found that SalvA itself induced robust c-Fos expression in limbic regions including the NAc shell, PfCx, LA, and CeA, suggesting an initiation of neuroplastic events in these regions that could underlie rodent correlates of the psychotropic effects of the drug. This was somewhat surprising, given the known inhibitory actions of KORs on intracellular signaling pathways and neurotransmitter release. In the striatum, KOR binding appears highest in the dorsomedial NAc shell (Unterwald *et al*, 1991), which is precisely where SalvA-induced c-Fos was observed in the current study. In the PfCx there is relatively little KOR binding, but KORs are thought to be on the cell bodies of dopaminergic neurons in the ventral tegmental area that project to the PfCx, where they act to inhibit DA cell firing (Margolis *et al*, 2006). In the amygdala, KOR binding and mRNA levels are high in the LA and low in the CeA (Unterwald *et al*, 1991; Mansour *et al*, 1994), although electrophysiological studies have shown that activation of KORs in the CeA results in inhibition of CeA neurons (Chieng *et al*, 2006), suggesting the presence of functional postsynaptic receptors in this region. It is also possible that KORs are expressed on presynaptic afferents in the amygdala. Thus, the ability of SalvA to induce c-Fos in these limbic brain regions most likely occurs through multiple direct and indirect mechanisms.

In conclusion, acute activation of KORs decreased behavioral and molecular responses to cocaine, and exposure to repeated activation of KORs altered the effects of cocaine in a context-dependent manner. The ability of acute SalvA to reduce presynaptic DA function in the dorsal striatum (Zhang *et al*, 2005) and NAc (Carlezon *et al*, 2006) may contribute to its ability to attenuate the acute stimulant properties of cocaine, raising the possibility that KOR agonists might be useful in the treatment of clinical conditions associated with elevated DA function in these regions (eg mania; see (Cohen and Murphy, 2007). However, chronic SalvA appears to cause changes in postsynaptic

(D1 receptor-related) signaling, and this effect may contribute to increased sensitivity to the stimulant effects of cocaine. Future studies examining the effects of acute and chronic SalvA on the reward-related effects of cocaine will be important, because they may provide an initial indication of whether repeated SalvA use in humans could alter vulnerability to addictive disorders. Regardless, these studies identify significant overlap in the molecular consequences of repeated exposure to stress, drugs of abuse, and KOR agonists.

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DISCLOSURE/CONFLICT OF INTEREST

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REFERENCES

Arvidsson U, Riedl M, Chakrabarti S, Vulchanova L, Lee JH, Nakano AH *et al* (1995). The kappa-opioid receptor is primarily postsynaptic: combined immunohistochemical localization of the receptor and endogenous opioids. *Proc Natl Acad Sci USA* **92**: 5062–5066.

Badiani A, Anagnostaras SG, Robinson TE (1995). The development of sensitization to the psychomotor stimulant effects of amphetamine is enhanced in a novel environment. *Psychopharmacology (Berl)* **117**: 443–452.

Badiani A, Oates MM, Day HEW, Watson SJ, Akil H, Robinson TE (1998). Amphetamine-induced behavior, dopamine release, and c-fos mRNA expression: modulation by environmental novelty. *J Neurosci* **18**: 10579–10593.

Badiani A, Robinson TE (2004). Drug-induced neurobehavioral plasticity: the role of environmental context. *Behav Pharmacol* **15**: 327–339.

Barrett JE (ed) (1987). *Nonpharmacological Factors Determining the Behavioral Effects of Drugs*. Raven: New York, pp 1493–1501.

Carlezon Jr WA, Beguin C, Dinieri JA, Baumann MH, Richards MR, Todtenkopf MS *et al* (2006). Depressive-like effects of the kappa-opioid receptor agonist salvinorin A on behavior and neurochemistry in rats. *J Pharmacol Exp Ther* **316**: 440–447.

Chavkin C, James IF, Goldstein A (1982). Dynorphin is a specific endogenous ligand of the kappa opioid receptor. *Science* **215**: 413–415.

Chavkin C, Sud S, Jin W, Stewart J, Zjawiony JK, Siebert DJ *et al* (2004). Salvinorin A, an active component of the hallucinogenic sage *Salvia divinorum* is a highly efficacious kappa-opioid receptor agonist: structural and functional considerations. *J Pharmacol Exp Ther* **308**: 1197–1203.

Chefer VI, Czyzyk T, Bolan EA, Moron J, Pintar JE, Shippenberg TS (2005). Endogenous kappa-opioid receptor systems regulate mesoaccumbal dopamine dynamics and vulnerability to cocaine. *J Neurosci* **25**: 5029–5037.

Chieng BC, Christie MJ, Osborne PB (2006). Characterization of neurons in the rat central nucleus of the amygdala: cellular physiology, morphology, and opioid sensitivity. *J Comp Neurol* **497**: 910–927.

Cohen BM, Murphy B (2007). The effects of pentazocine, a kappa agonist, in patients with mania. *Int J Neuropsychopharmacol* 1–5. (E-pub ahead of print 26 September 2007).

Covington III HE, Miczek KA (2001). Repeated social-defeat stress, cocaine or morphine. Effects on behavioral sensitization and intravenous cocaine self-administration 'binges'. *Psychopharmacology (Berl)* **158**: 388–398.

Di Chiara G, Imperato A (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci USA* **85**: 5274–5278.

Erb S, Shaham Y, Stewart J (1996). Stress reinstates cocaine-seeking behavior after prolonged extinction and a drug-free period. *Psychopharmacology (Berl)* **128**: 408–412.

Falk JL, Feingold DA (eds). (1987). *Environmental and Cultural Factors in the Behavioral Actions of Drugs*. Raven: New York, pp 1503–1510.

Goussakov I, Chartoff EH, Tsvetkov E, Gerety LP, Meloni EG, Carlezon Jr WA *et al* (2006). LTP in the lateral amygdala during cocaine withdrawal. *Eur J Neurosci* **23**: 239–250.

Gray AM, Rawls SM, Shippenberg TS, McGinty JF (1999). The kappa-opioid agonist, U-69593, decreases acute amphetamine-evoked behaviors and calcium-dependent dialysate levels of dopamine and glutamate in the ventral striatum. *J Neurochem* **73**: 1066–1074.

Harlan RE, Garcia MM (1998). Drugs of abuse and immediate-early genes in the forebrain. *Mol Neurobiol* **16**: 221–267.

Heidbreder CA, Babovic-Vuksanovic D, Shoaib M, Shippenberg TS (1995). Development of behavioral sensitization to cocaine: influence of kappa opioid receptor agonists. *J Pharmacol Exp Ther* **275**: 150–163.

Heidbreder CA, Schenk S, Partridge B, Shippenberg TS (1998). Increased responsiveness of mesolimbic and mesostriatal dopamine neurons to cocaine following repeated administration of a selective kappa-opioid receptor agonist. *Synapse* **30**: 255–262.

Herman JP, Stinus L, Le Moal M (1984). Repeated stress increases locomotor response to amphetamine. *Psychopharmacology (Berl)* **84**: 431–435.

Hjelmstad GO, Fields HL (2003). Kappa opioid receptor activation in the nucleus accumbens inhibits glutamate and GABA release through different mechanisms. *J Neurophysiol* **89**: 2389–2395.

Hurd YL, Brown EE, Finlay JM, Fibiger HC, Gerfen CR (1992). Cocaine self-administration differentially alters mRNA expression of striatal peptides. *Brain Res Mol Brain Res* **13**: 165–170.

Izenwasser S, Acri JB, Kunko PM, Shippenberg T (1998). Repeated treatment with the selective kappa opioid agonist U-69593 produces a marked depletion of dopamine D2 receptors. *Synapse* **30**: 275–283.

Kessler RC (1997). The effects of stressful life events on depression. *Annu Rev Psychol* **48**: 191–214.

Kiyatkin EA (1992). State-dependent peculiarities of cocaine-induced behavioral sensitization and their possible reasons. *Int J Neurosci* **67**: 93–103.

Koob GF, Le Moal M (1997). Drug abuse: hedonic homeostatic dysregulation. *Science* **278**: 52–58.

Koob GF, Stinus L, Le Moal M, Bloom FE (1989). Opponent process theory of motivation: neurobiological evidence from studies of opiate dependence. *Neurosci Biobehav Rev* **13**: 135–140.

Kuzmin AV, Gerrits M, Van Ree JM (1998). kappa-opioid receptor blockade with nor-binaltorphimine modulates cocaine self-administration in drug-naive rats. *Eur J Pharmacol* **358**: 197–202.

Lee DY, Ma Z, Liu-Chen LY, Wang Y, Chen Y, Carlezon Jr WA *et al* (2005). New neoclerodane diterpenoids isolated from the leaves of *Salvia divinorum* and their binding affinities for human kappa opioid receptors. *Bioorg Med Chem* **13**: 5635–5639.

Lu L, Shepard JD, Scott Hall F, Shaham Y (2003). Effect of environmental stressors on opiate and psychostimulant reinforcement, reinstatement and discrimination in rats: a review. *Neurosci Biobehav Rev* **27**: 457–491.

Mansour A, Fox CA, Burke S, Meng F, Thompson RC, Akil H *et al* (1994). Mu, delta, and kappa opioid receptor mRNA expression in the rat CNS: an *in situ* hybridization study. *J Comp Neurol* **350**: 412–438.

Margolis EB, Lock H, Chefer VI, Shippenberg TS, Hjelmstad GO, Fields HL (2006). Kappa opioids selectively control dopaminergic neurons projecting to the prefrontal cortex. *Proc Natl Acad Sci USA* **103**: 2938–2942.

Markou A, Kosten TR, Koob GF (1998). Neurobiological similarities in depression and drug dependence: a self-medication hypothesis. *Neuropsychopharmacology* **18**: 135–174.

McDaniel KL, Mundy WR, Tilson HA (1990). Microinjection of dynorphin into the hippocampus impairs spatial-learning in rats. *Pharmacol Biochem Behav* **35**: 429–435.

McLaughlin JP, Land BB, Li S, Pintar JE, Chavkin C (2006). Prior activation of kappa opioid receptors by U50,488 mimics repeated forced swim stress to potentiate cocaine place preference conditioning. *Neuropsychopharmacology* **31**: 787–794.

McLaughlin JP, Marton-Popovici M, Chavkin C (2003). Kappa opioid receptor antagonism and prodynorphin gene disruption block stress-induced behavioral responses. *J Neurosci* **23**: 5674–5683.

McLaughlin JP, Myers LC, Zarek PE, Caron MG, Lefkowitz RJ, Czyzyk TA *et al* (2004). Prolonged kappa opioid receptor phosphorylation mediated by G-protein receptor kinase underlies sustained analgesic tolerance. *J Biol Chem* **279**: 1810–1818.

Mello NK, Negus SS (2000). Interactions between kappa opioid agonists and cocaine. Preclinical studies. *Ann N Y Acad Sci* **909**: 104–132.

Meshul CK, McGinty JF (2000). Kappa opioid receptor immunoreactivity in the nucleus accumbens and caudate-putamen is primarily associated with synaptic vesicles in axons. *Neuroscience* **96**: 91–99.

Morgan JI, Curran T (1991). Stimulus-transcription coupling in the nervous-system—involvement of the inducible protooncogenes Fos and Jun. *Annu Rev Neurosci* **14**: 421–451.

Munro TA, Rizzacasa MA, Roth BL, Toth BA, Yan F (2005). Studies toward the pharmacophore of salvinorin A, a potent kappa opioid receptor agonist. *J Med Chem* **48**: 345–348.

Negus SS (2004). Effects of the kappa opioid agonist U50,488 and the kappa opioid antagonist nor-binaltorphimine on choice between cocaine and food in rhesus monkeys. *Psychopharmacology (Berl)* **176**: 204–213.

Negus SS, Mello NK, Portoghese PS, Lin CE (1997). Effects of kappa opioids on cocaine self-administration by rhesus monkeys. *J Pharmacol Exp Ther* **282**: 44–55.

Paxinos G, Watson C (1986). *The Rat Brain in Stereotaxic Coordinates*. Academic Press: New York.

Pfeiffer A, Brantl V, Herz A, Emrich HM (1986). Psychotomimesis mediated by kappa opiate receptors. *Science* **233**: 774–776.

Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ (1987). Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science* **237**: 1219–1223.

Robinson TE, Berridge KC (1993). The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Brain Res Rev* **18**: 247–291.

Roth BL, Baner K, Westkaemper R, Siebert D, Rice KC, Steinberg S *et al* (2002). Salvinorin A: a potent naturally occurring nonnitrogenous kappa opioid selective agonist. *Proc Natl Acad Sci USA* **99**: 11934–11939.

Schenk S, Partridge B, Shippenberg TS (1999). U69593, a kappa-opioid agonist, decreases cocaine self-administration and decreases cocaine-produced drug-seeking. *Psychopharmacology (Berl)* **144**: 339–346.

Shaham Y, Shalev U, Lu L, de Wit H, Stewart J (2003). The reinstatement model of drug relapse: history, methodology and major findings. *Psychopharmacology (Berl)* **168**: 3–20.

Sheng M, McFadden G, Greenberg ME (1990). Membrane depolarization and calcium induce c-fos transcription via phosphorylation of transcription factor CREB. *Neuron* **4**: 571–582.

Shippenberg TS, Herz A (1988). Motivational effects of opioids: influence of D-1 versus D-2 receptor antagonists. *Eur J Pharmacol* **151**: 233–242.

Shippenberg TS, LeFevour A, Heidbreder C (1996). kappa-Opioid receptor agonists prevent sensitization to the conditioned rewarding effects of cocaine. *J Pharmacol Exp Ther* **276**: 545–554.

Shippenberg TS, Rea W (1997). Sensitization to the behavioral effects of cocaine: modulation by dynorphin and kappa-opioid receptor agonists. *Pharmacol Biochem Behav* **57**: 449–455.

Shirayama Y, Ishida H, Iwata M, Hazama GI, Kawahara R, Duman RS (2004). Stress increases dynorphin immunoreactivity in limbic brain regions and dynorphin antagonism produces antidepressant-like effects. *J Neurochem* **90**: 1258–1268.

Siebert DJ (1994). *Salvia divinorum* and salvinorin A: new pharmacologic findings. *J Ethnopharmacol* **43**: 53–56.

Sinha R, Catapano D, O'Malley S (1999). Stress-induced craving and stress response in cocaine dependent individuals. *Psychopharmacology (Berl)* **142**: 343–351.

Smiley PL, Johnson M, Bush L, Gibb JW, Hanson GR (1990). Effects of cocaine on extrapyramidal and limbic dynorphin systems. *J Pharmacol Exp Ther* **253**: 938–943.

Spanagel R, Herz A, Shippenberg TS (1992). Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. *Proc Natl Acad Sci USA* **89**: 2046–2050.

Spangler R, Unterwald EM, Kreek MJ (1993). 'Binge' cocaine administration induces a sustained increase of prodynorphin mRNA in rat caudate-putamen. *Brain Res Mol Brain Res* **19**: 323–327.

Svingos AL, Colago EE, Pickel VM (1999). Cellular sites for dynorphin activation of kappa-opioid receptors in the rat nucleus accumbens shell. *J Neurosci* **19**: 1804–1813.

Thompson AC, Zapata A, Justice Jr JB, Vaughan RA, Sharpe LG, Shippenberg TS (2000). Kappa-opioid receptor activation modifies dopamine uptake in the nucleus accumbens and opposes the effects of cocaine. *J Neurosci* **20**: 9333–9340.

Todtenkopf MS, Carlezon Jr WA (2006). Contribution of drug doses and conditioning periods to psychomotor stimulant sensitization. *Psychopharmacology (Berl)* **185**: 451–458.

Todtenkopf MS, Marcus JF, Portoghese PS, Carlezon Jr WA (2004). Effects of kappa-opioid receptor ligands on intracranial self-stimulation in rats. *Psychopharmacology (Berl)* **172**: 463–470.

Unterwald EM, Knapp C, Zukin RS (1991). Neuroanatomical localization of kappa-1 and kappa-2 opioid receptors in rat and guinea-pig brain. *Brain Res* **562**: 57–65.

Wise RA, Bozarth MA (1987). A psychomotor stimulant theory of addiction. *Psychol Rev* **94**: 469–492.

Yan F, Roth BL (2004). Salvinorin A: a novel and highly selective kappa opioid receptor agonist. *Life Sci* **75**: 2615–2619.

Zhang Y, Butelman ER, Schlussman SD, Ho A, Kreek MJ (2005). Effects of the plant-derived hallucinogen salvinorin A on basal dopamine levels in the caudate putamen and in a conditioned place aversion assay in mice: agonist actions at kappa opioid receptors. *Psychopharmacology (Berl)* **179**: 551–558.